BERKELEY LAB
LAWRENCE BERKELEY NATIONAL LABORATORY

LIFE SCIENCES DIVISION E-NEWSLETTER

July/August, 2008

In this issue:

DOE scientific focus area notes

Low Dose Radiation Research

Scientific Retreat for DOE Low Dose SFA at Berkeley Lab
The members of the DOE Scientific Focus Area on Low Dose Radiation Research, headed by **Joe Gray**, will attend an all day scientific retreat on September 8, 2008. The program will include discussions by leaders of the Adaptive Response Team (**Priscilla Cooper**, **Andrew Wyrobek**), the Cancer Genetics Team (**Jian-Hua Mao**, **Mina Bissell**), and the Epigenetics Team (**Terumi Kohwi-Shigematsu**, **Gary Karpen**). The meeting will include a poster session of presentations by young investigators and team scientists.
Joe Gray, 8/08

DOE-Sponsored Scientific Symposium on Low Dose Radiation-Induced Genome and Epigenome Instability at Upcoming EMS Annual Meeting
The Environmental Mutagen Society (EMS-US) will host its annual scientific conference in San Juan Puerto Rico, October 18-24, 2008 (http://www.ems-us.org/AM2008/). **Priscilla Cooper** is program chair and president-elect of the EMS-US. The theme of the meeting is "Genes and the Environment: From Molecular Mechanisms to Risk." The five day meeting will provide a forum for integrating cutting-edge basic research in DNA repair, mechanisms of mutagenesis, and epigenetic regulation in response to environmental genotoxic agents with translational research, risk assessment, and regulatory concerns. The conference is sponsored in part by the US Department of Energy Low Dose Program. **Andrew Wyrobek** of the Life Sciences Division is the current president of the society.
Radiation Biosciences Department, 8/08

GTL-Genomics

Particle Detection in Electron Microscopy Images
One of the goals of the Protein Complex Analysis Project (PCAP) is to derive models for the major macromolecular complexes in Desulfovibrio vulgaris that can be used as templates in analyzing electron tomograms of cells. The approach taken in the PCAP project is to record electron micrographs of purified complexes and use computer processing to arrive at the 3-D structure, with a target resolution of 15-30 Å. Because of the noisy nature of electron micrographs of proteins at this resolution, large numbers of individual particle images need to be combined, and the number increases rapidly as the target resolution becomes more ambitious. The current target requires several thousand particles, while reaching "atomic" resolution may require closer to a million. Identifying the particles in a micrograph is a major bottleneck for a high throughput pipeline such as PCAP. **Bong-Gyoon Han** and **Bob Glaeser** have already studied over a dozen complexes from D. vibrio in work that has required the hand selection of tens of thousands of particles. As part of PCAP, Pablo Arbeláez and Jitendra Malik have been using modern image analysis methods to develop a general software package for automatic detection of particles and break this bottleneck.

Their approach treats particle picking as a visual pattern recognition problem in which a few examples of a particle are used to detect new instances of the particle in a whole micrograph. The method uses texture information as the main perceptual cue to differentiate molecular structures from background. A multi-stage process has been developed to implement the selection. In the first stage, the system builds a model for the texture of a collection of hand-selected particles. This stage results in a collection of textons, which allows expressing the information of any image with respect to the texture of the examples. An example is presented in Figure 1 a-b below.

In the second stage of processing, the system builds an appearance model for the particles by considering the texture distributions within and in surrounding areas that are the size of the particle. This allows improved discrimination of isolated particles from other features.

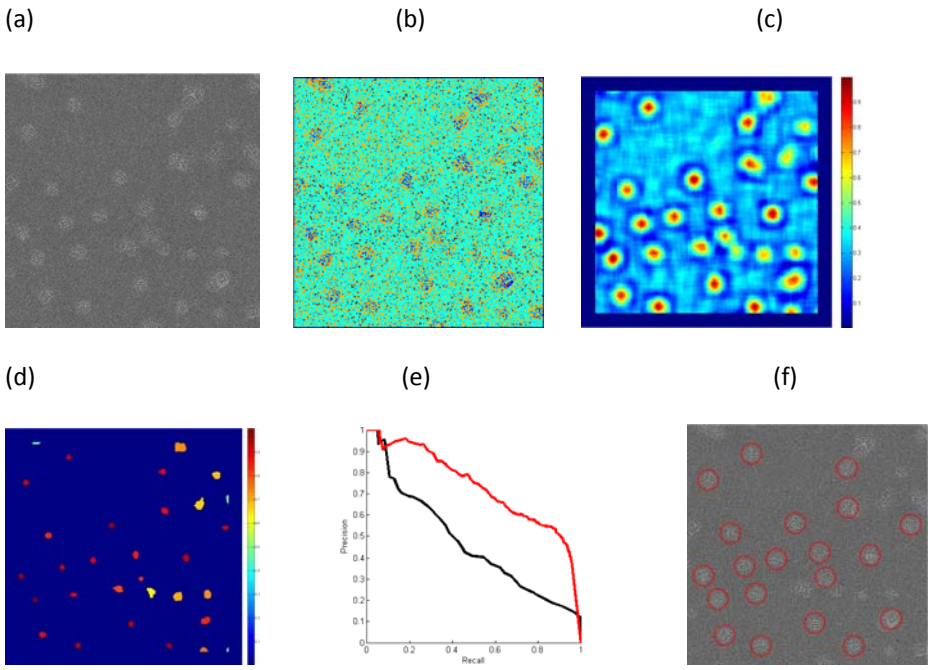


Figure 1: **(a):** Original micrograph of GroEl protein complexes. **(b):** texton map. **(c)**: SVM probability. **(d):** detections weighted by confidence. **(e):** Precision-Recall curve of this method (red) compared to a baseline approach using cross-correlation (black). **(f):** Final result of this method.

Next, the candidate particles and other features are represented in a multidimensional space, and a discriminative learning technique called Support Vector Machine (SVM) is used to classify the data. This results in a map such as Fig. 1c which can be used to assign locations of particles and the confidence of their proper detection (Fig. 1d). The performance of the system is evaluated by considering its precision, defined as the fraction of true positives among the computer's detections, and recall, defined as the fraction of detections among the human's (Fig. 1e), to capture the tradeoff between false positives and missed detections as the confidence measure varies. The final result is obtained by choosing a threshold on the confidence. While a number of other automated schemes for selecting

particles in electron micrographs have been presented, each has its own limitations. The combination of state-of-the-art image analysis routines tailored to the characteristics of protein images should make this approach a particularly useful tool in our high-throughput efforts.
Kenneth Downing, 7/08

Nuclear Medicine

Transition Celebration for Budinger

Thomas Budinger

A transition celebration for **Thomas Budinger** was held on August 12, 2008 at Berkeley Lab. The celebration marks Budinger's retirement and transition to part time rehire. A number of colleagues spoke of his extensive and eclectic career as an oceanographer, physician, biophysicist, mentor, educator, author, and world-class scientist. He will be rehired on a part-time basis to help develop new research in support of DOE's missions in biofuels and environmental remediation. Budinger joined the staff of the Donner Laboratory and Lawrence Radiation Laboratory Berkeley's Medical Services group at Berkeley Lab as research scientist and staff physician in 1968. On the same day of the celebration meetings were held with Prem Srivastava of DOE's Office of Biological and Environmental Research to discuss how Berkeley Lab's expertise in radiochemistry and imaging instrumentation can continue to support their mission.
Stephen Derenzo, 8/08

Scientific news

Understanding Hearing, Molecule by Molecule
Berkeley Lab scientists have for the first time pieced together the three-dimensional structure of one of nature's most exquisite pieces of machinery, a gossamer-like filament of proteins in the inner ear that enables the sense of hearing and balance. This work of life scientist **Manfred Auer** and colleagues opens the door for a more fundamental understanding of how hearing works. It may also lead to improved ways to treat some forms of hearing loss, which affects about ten percent of people. [More]
http://www.lbl.gov/publicinfo/newscenter/features/2008/LS-ear.html
Today at Berkeley Lab, 7/11/08

Life Scientist Discusses XPD Protein on Podcast

Jill Fuss

Life Scientist **Jill Fuss**, working in the laboratories of **Priscilla Cooper** and **John Tainer**, helped solve the structure of the important DNA repair protein XPD, as reported in a recent article in *Cell*. In a Podcast interview with Genetic Engineering and Biotechnology News, Fuss discusses how XPD can contribute to understanding cancer and aging.
http://www.genengnews.com/wimpy/gcn_interview.aspx?gcn_id=218
Today at Berkeley Lab, 7/14/08

Sudar Presents at Microscopy Society of America Conference

In a special pre-meeting congress on Cellular Analysis: Linking Quantitation to Structure and Function, life scientist **Damir Sudar** gave an invited talk on high-throughput quantitative 3D imaging to construct a spatio-temporal atlas of morphology and gene expression in a complex animal. This congress was part of the annual Microscopy and Microanalysis meeting of the Microscopy Society of America in Albuquerque, NM on August 2-7, 2008. An integrated team of Berkeley Lab and university (University of California at Berkeley and Davis) researchers have conducted the work presented. Some of the Berkeley Lab scientists on the team are **Mark Biggin**, **David Knowles**, **Gunther Weber**, and **Michael Eisen**.

Damir Sudar, 08/08

Life Scientists Present Work at Microscopy and Microanalysis Meeting

Life scientist **Dieter Typke** presented an invited talk at the Microscopy and Microanalysis Meeting (August 2-7, 2008), "Aspects of using a Boersch-type phase shifting device for contrast enhancement in macromolecular electron microscopy". He presented some results from the work on **Robert Glaeser**'s project, which also includes **Kenneth Downing**'s work as well as that of Rossana Cambie and Jian Jin from Berkeley Lab's Engineering Division. Glaeser gave a talk on "Proteomic survey of large macromolecular complexes in D. vulgaris" - work of the PCAP project that also includes **Bong Gyoon-Han** and Typke.

Kenneth Downing, 8/08

Lab Life Scientist's Nature Paper on Postponing Aging

Research on lab organisms such as yeast, worms and mice has encouraged the notion that lifespan can be manipulated genetically. The big question is whether this work translates to the human condition. In a *Nature* review, Jan Vijg and Berkeley Lab life scientist **Judith Campisi** discuss current knowledge about factors that limit human lifespan. They conclude that it is too early to say whether it is feasible to postpone human aging and natural death for many decades.

http://www.nature.com/nature/journal/v454/n7208/edsumm/e080828-05.html

Today at Berkeley Lab, 08/29/08

Blakely Scientific Organizer and Presenter on "Radiobiology of Particles"

Eleanor Blakely of the Life Sciences Division was the scientific organizer of the session on "Radiobiology of Particles" at the 20th International Conference on the Application of Accelerators in Research & Industry (CAARI) held in Ft. Worth, Texas, 10-15 August 2008. She made three oral presentations, one of which was videotaped for distribution by CAARI, one in the session she organized (an overview), and one in a summary session. CAARI brings together scientists from all over the world who use particle accelerators in their research and industrial applications.

Radiation Biosciences Department, 8/08

Radiation-Induced Genomic Instability in Finite Lifespan HMEC

The July 2008 issue of *Radiation Research* features a research paper from the laboratory of life scientist **Amy Kronenberg** on the topic of radiation-induced genomic instability in finite lifespan human mammary epithelial cells (HMEC) (Sudo, et al., *Rad. Res.* 170:23-32, 2008). This paper is the first to demonstrate radiation-induced genomic instability in non-immortalized, non-tumorigenic human epithelial cells and improves our understanding of the high sensitivity of the female human breast to radiation-induced carcinogenesis. The study examined the relative risks of karyotypic instability and centrosome aberrations in the progeny of individual cells exposed to sparsely ionizing x-rays or densely ionizing Fe ions. Each radiation type increased the risk of persistent genomic instability to very high frequencies (approximately 10%) that are not consistent with a simple mutational hypothesis. The results suggest that radiation exposures induced persistent phenotypic changes in surviving HMECs that may lead to non-clonal chromosomal rearrangements. This effect was more prevalent after exposure to densely ionizing radiation than sparsely ionizing radiation. The team contributing to this research included **Hiroko Sudo**, **Martha Stampfer**, **James Garbe**, **Mary Helen Barcellos-Hoff**, and **Amy Kronenberg**. This work was supported by the NASA NSCOR in Radiation Health at Berkeley Lab.
Radiation Biosciences Department, 8/08

Life Sciences Researchers Host of Several CSEE Undergraduate Summer Interns

CSEE Berkeley Lab's Center for Science and Engineering Education (CSEE) held the annual Undergraduate Summer Intern Poster Session on August 11, 2008. This event is one of the rare opportunities to bring together, at one time, examples of the breadth of research conducted at the Lab, through the experiences of the students who have been at the Lab this summer participating in CSEE mentored internship programs. Several students worked under the mentorship of Life Sciences researchers.

Stephen Derenzo with intern

Alicia Gunterus, a student sponsored by the DOE Office of Science Community College Institute program, was mentored by **Tamas Torok**. Gunterus worked on a biofilm that was collected earlier at an extreme environmental site on the Kamchatka peninsula in the Russian Far-East. She used DNA microarray analysis to characterize the structure and function of the microbial community that formed the biofilm and confirmed this information by scanning and transmission electron microscopy, a project in collaboration with the **Auer** laboratory and the Andersen laboratory of the Earth Sciences Division. Gunterus's paper on her intern work was accepted for the *DOE Undergraduate Research Journal*.

Steve Yannone with intern

MAGGIE, the Life Sciences GTL program project, hosted several students. They worked on the isolation of intact membrane protein complexes from the extremophilic Archaea Sulfolobus solfataricus which lives at 80 degrees centigrade and a pH of 2-3. Brandon Sheppard from Corpus Christi Texas presented his work on the use of dynamic light scattering to detect the dissolution of membrane vesicles at various detergent concentrations. **Steve Yannone** commented: "It is always a gratifying experience to challenge bright students with scientific problems and help them grapple with them and ultimately come to some answers and understanding."

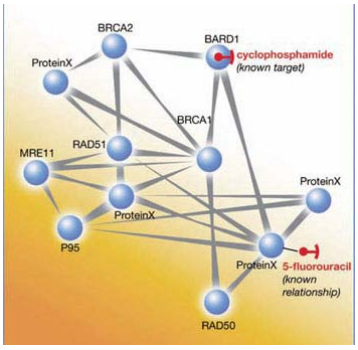
Trey Jalbert and Carolyn Posey joined **Eleanor Blakely**'s lab. Jalbert worked on the NASA grant studying MMPs in human lens epithelial cells (HLE) and was able to travel with the research team to Brookhaven National Lab, as a replacement team member, to irradiate HLE cells at the NASA Space Research Laboratory (NSRL), Brookhaven, NY. Posey, a senior at Creighton University, worked on Blakely's Low Dose DOE grant studying the total TP53 and TP53^{ser15}responses of human mammary epithelial cells in and out of 6 and 11 um stripes of microbeam doses.
CG, Today at Berkeley Lab, 8/11/08

Awards

Jagust to Win Zenith Fellow Award
William Jagust has been awarded a Zenith Fellows award from the Alzheimer's Association, initiated in 1991 to provide a vehicle for research support for donors with a substantial personal commitment to the advancement of Alzheimer's disease research. The award is one of five awards made possible by the generosity of a group of individuals and organizations (Zenith Fellows) who have each committed $1 million to the Alzheimer's Association for support of the program. The objective of the Zenith Awards competition is to provide major support for investigators who have contributed significantly to the field of Alzheimer's disease research.

Jagust's proposed research was selected for being "on the cutting edge" of basic science or biomedical research addressing fundamental problems related to early detection, etiology, pathogenesis, treatment, and prevention of Alzheimer's disease. The $450,000 award for three years will fund a project designed to detect evidence of early Alzheimer's disease in cognitively normal, healthy older people by using PET scanning for the presence of beta-amyloid, the protein that is thought to be responsible for the disease.
CG, 08/08

Life Sciences Wins Award for Technology Advances
Four of *R&D Magazine*'s prestigious R&D 100 Awards for 2008, which recognize the 100 most significant proven technological advances of the year, have gone to researchers at Berkeley Lab and their colleagues. The innovations include the Berkeley Lab PhyloChip, Biomimetic Search Engine, FastBit Bitmap Index, and Nanostructured Polymer Electrolyte for Rechargeable Lithium Batteries.



Biomimetic Search Engine result

The Biomimetic Search Engine is the only search engine that couples the way people learn with the unmatched speed and data storage capabilities of computers. In doing so, it is revolutionizing how digital content is searched and utilized. Users can search huge databases and determine how objects are related, in what contexts they are related, and the strengths of those relationships. It was developed by **Kasian Franks** and **Connie Myers**, formerly of Berkeley Lab's Life Sciences Division, and Raf Podowski. Both Franks and Podowski are now with the Emeryville, CA-based start-up company SeeqPod. A music search

engine using the Biomimetic Search Engine can be found at www.seeqpod.com.

The Biomimetic Search Engine goes far beyond simply sifting through data for keywords. Instead, the system allows users to make previously unknown connections between seemingly unrelated terms. In this way, it helps to synthesize new information and further a person's understanding of a given topic, which is the hallmark of discovery, innovation, and invention. The Biomimetic Search Engine is already revolutionizing how genomics data, the internet's playable music and video files, and Wikipedia's ever-growing content are searched and utilized. It is also poised to make inroads into the finance, sports, and health sectors. <More> http://www.lbl.gov/publicinfo/newscenter/pr/2008/TT-RD100.html
Today at Berkeley Lab, 7/9/08

Kronenberg Appointed to Governing Council of Radiation Research Society
Amy Kronenberg was elected the serve on a Governing Council of the Radiation Research Society. The Society's objectives are to encourage in the broadest manner the advancement of radiation research in all areas of the natural sciences; To facilitate cooperative research between the disciplines of physics, chemistry, biology and medicine in the study of the properties and effects of radiation; and to promote dissemination of knowledge in these and related fields through publications, meetings and educational symposia. [More about the Society]
http://www.radres.org/ECOMradres/timssnet/common/tnt_frontpage.cfm
Radiation Biosciences Department, 8/08

Recent publications (selected)

Martin KJ, Patrick DR, **Bissell MJ**, Fournier MV. Prognostic breast cancer signature identified from 3D culture model accurately predicts clinical outcome across independent datasets. *PLoS ONE*, 2008 Aug 20;3(8):e2994. PMID: 18714348

> BACKGROUND: One of the major tenets in breast cancer research is that early detection is vital for patient survival by increasing treatment options. To that end, we have previously used a novel unsupervised approach to identify a set of genes whose expression predicts prognosis of breast cancer patients. The predictive genes were selected in a well-defined three dimensional (3D) cell culture model of non-malignant human mammary epithelial cell morphogenesis as down-regulated during breast epithelial cell acinar formation and cell cycle arrest. Here we examine the ability of this gene signature (3D-signature) to predict prognosis in three independent breast cancer microarray datasets having 295, 286, and 118 samples, respectively. METHODS AND FINDINGS: Our results show that the 3D-signature accurately predicts prognosis in three unrelated patient datasets. At 10 years, the probability of positive outcome was 52, 51, and 47 percent in the group with a poor-prognosis signature and 91, 75, and 71 percent in the group with a good-prognosis signature for the three datasets, respectively (Kaplan-Meier survival analysis, $p<0.05$). Hazard ratios for poor outcome were 5.5 (95% CI 3.0 to 12.2, $p<0.0001$), 2.4 (95% CI 1.6 to 3.6, $p<0.0001$) and 1.9 (95% CI 1.1 to 3.2, $p = 0.016$) and remained significant for the two larger datasets when corrected for estrogen receptor (ER) status. Hence the 3D-signature accurately predicts breast cancer outcome in both ER-positive and ER-negative tumors, though individual genes differed in their prognostic ability in the two subtypes. Genes that were prognostic in ER+ patients are AURKA, CEP55, RRM2, EPHA2, FGFBP1, and VRK1, while genes prognostic in ER- patients include ACTB, FOXM1 and SERPINE2 (Kaplan-Meier $p<0.05$). Multivariable Cox regression analysis in the largest dataset showed that the 3D-signature

was a strong independent factor in predicting breast cancer outcome. CONCLUSIONS: The 3D-signature accurately predicts breast cancer outcome across multiple datasets and holds prognostic value for both ER-positive and ER-negative breast cancer. The signature was selected using a novel biological approach and hence holds promise to represent the key biological processes of breast cancer.

Holman HY, Bjornstad KA, Martin MC, McKinney WR, **Blakely EA**, Blankenberg FG. Mid-infrared reflectivity of experimental atheromas. *Journal of Biomedical Optics*, 2008 May-Jun;13(3):030503. PMID: 18601520

We report that the pathologic components present within the atheromatous plaques of ApoE knock-out mice can reflect significant amounts of mid-infrared (mid-IR) light. Furthermore, the reflected light spectra contained the unique signatures of a variety of biologic features including those found in unstable or "vulnerable" plaque. This discovery may represent a unique opportunity to develop a new intravascular diagnostic modality that can detect and characterize sites of atherosclerosis.

Coppe JP, Boysen M, Sun CH, Wong BJ, Kang MK, Park NH, Desprez PY, **Campisi J**, Krtolica A. A role for fibroblasts in mediating the effects of tobacco-induced epithelial cell growth and invasion. *Molecular Cancer Research*, 2008 Jul;6(7):1085-98. PMID: 18644973

Cigarette smoke and smokeless tobacco extracts contain multiple carcinogenic compounds, but little is known about the mechanisms by which tumors develop and progress upon chronic exposure to carcinogens such as those present in tobacco products. Here, we examine the effects of smokeless tobacco extracts on human oral fibroblasts. We show that smokeless tobacco extracts elevated the levels of intracellular reactive oxygen, oxidative DNA damage, and DNA double-strand breaks in a dose-dependent manner. Extended exposure to extracts induced fibroblasts to undergo a senescence-like growth arrest, with striking accompanying changes in the secretory phenotype. Using cocultures of smokeless tobacco extracts-exposed fibroblasts and immortalized but nontumorigenic keratinocytes, we further show that factors secreted by extracts-modified fibroblasts increase the proliferation and invasiveness of partially transformed epithelial cells, but not their normal counterparts. In addition, smokeless tobacco extracts-exposed fibroblasts caused partially transformed keratinocytes to lose the expression of E-cadherin and ZO-1, as well as involucrin, changes that are indicative of compromised epithelial function and commonly associated with malignant progression. Together, our results suggest that fibroblasts may contribute to tumorigenesis indirectly by increasing epithelial cell aggressiveness. Thus, tobacco may not only initiate mutagenic changes in epithelial cells but also promote the growth and invasion of mutant cells by creating a procarcinogenic stromal environment.

Vijg J and **Campisi J**. Puzzles, promises and a cure for ageing. *Nature,* 28 August 2008, 454, 1065-1071, doi:10.1038/nature07216

Editor's Summary: Research on lab organisms such as yeast, worms and mice has encouraged the notion that lifespan is plastic, something to be manipulated genetically, with drugs or by nutritional fine-tuning. The big question is how — or whether — this work translates to the human condition. In a review, Jan Vijg and Judith Campisi discuss current knowledge about factors that limit human lifespan. They conclude that it is too early to say whether it is feasible to postpone human ageing and natural death for many decades. And they outline questions that future research needs to answer if we are to develop integrated strategies capable of increasing human health and lifespan.

Pfeiffer BD, Jenett A, Hammonds AS, Ngo TT, Misra S, Murphy C, Scully A, Carlson JW, Wan KH, Laverty TR, Mungall C, Svirskas R, Kadonaga JT, Doe CQ, Eisen MB, **Celniker SE**, **Rubin GM**. Tools for

neuroanatomy and neurogenetics in Drosophila. *Proceedings of National Academy of Sciences USA*, 2008 Jul 15;105(28):9715-20. PMID: 18621688

> We demonstrate the feasibility of generating thousands of transgenic Drosophila melanogaster lines in which the expression of an exogenous gene is reproducibly directed to distinct small subsets of cells in the adult brain. We expect the expression patterns produced by the collection of 5,000 lines that we are currently generating to encompass all neurons in the brain in a variety of intersecting patterns. Overlapping 3-kb DNA fragments from the flanking noncoding and intronic regions of genes thought to have patterned expression in the adult brain were inserted into a defined genomic location by site-specific recombination. These fragments were then assayed for their ability to function as transcriptional enhancers in conjunction with a synthetic core promoter designed to work with a wide variety of enhancer types. An analysis of 44 fragments from four genes found that >80% drive expression patterns in the brain; the observed patterns were, on average, comprised of <100 cells. Our results suggest that the D. melanogaster genome contains >50,000 enhancers and that multiple enhancers drive distinct subsets of expression of a gene in each tissue and developmental stage. We expect that these lines will be valuable tools for neuroanatomy as well as for the elucidation of neuronal circuits and information flow in the fly brain.

Chang H, Defilippis RA, Tlsty TD, **Parvin B**. Graphical methods for quantifying macromolecules through bright field imaging. *Bioinformatics*, 2008 Aug 14. PMID: 18703588

> Bright field imaging of biological samples stained with antibodies and/or special stains provides a rapid protocol for visualizing various macromolecules. However, this method of sample staining and imaging is rarely employed for direct quantitative analysis due to variations in sample fixations, ambiguities introduced by color composition, and the limited dynamic range of imaging instruments. We demonstrate that, through the decomposition of color signals, staining is scored on a cell-by-cell basis. We applied our method to fibroblasts grown from histologically normal breast tissue biopsies obtained from two distinct populations. Firstly, nuclear regions are initially segmented through conversion of color images into gray scale, and detection of dark elliptic features. Subsequently, the strength of staining is quantified by a color decomposition model that is optimized by a graph cut algorithm. In rare cases where nuclear signal is significantly altered as a result of sample preparation, nuclear segmentation can be validated and corrected. Finally, stained patterns are associated with each nuclear region following region-based tessellation. Compared to classical non-negative matrix factorization, our new proposed method (i) improves color decomposition, (ii) has a better noise immunity, (iii) is more invariant to initial conditions, and (iv) has a superior computing performance.

Barkan D, Kleinman H, Simmons JL, Asmussen H, Kamaraju AK, Hoenorhoff MJ, Liu ZY, **Costes SV**, Cho EH, Lockett S, Khanna C, Chambers AF, Green JE. Inhibition of metastatic outgrowth from single dormant tumor cells by targeting the cytoskeleton. *Cancer Research*, 2008 Aug 1; 68(15):6241-50. PMID: 18676848

> Metastatic breast cancer may emerge from latent tumor cells that remain dormant at disseminated sites for many years. Identifying mechanisms regulating the switch from dormancy to proliferative metastatic growth has been elusive due to the lack of experimental models of tumor cell dormancy. We characterized the in vitro growth characteristics of cells that exhibit either dormant (D2.0R, MCF-7, and K7M2AS1.46) or proliferative (D2A1, MDA-MB-231, and K7M2) metastatic behavior in vivo. Although these cells proliferate readily in two-dimensional culture, we show that when grown in three-dimensional matrix, distinct growth properties of the cells were revealed that correlate to their dormant or proliferative behavior at metastatic sites in vivo. In three-dimensional culture, cells with dormant behavior in vivo remained cell cycle arrested with elevated nuclear expression of p16 and p27. The

transition from quiescence to proliferation of D2A1 cells was dependent on fibronectin production and signaling through integrin beta1, leading to cytoskeletal reorganization with filamentous actin (F-actin) stress fiber formation. We show that phosphorylation of myosin light chain (MLC) by MLC kinase (MLCK) through integrin beta1 is required for actin stress fiber formation and proliferative growth. Inhibition of integrin beta1 or MLCK prevents transition from a quiescent to proliferative state in vitro. Inhibition of MLCK significantly reduces metastatic outgrowth in vivo. These studies show that the switch from dormancy to metastatic growth may be regulated, in part, through epigenetic signaling from the microenvironment, leading to changes in the cytoskeletal architecture of dormant cells. Targeting this process may provide therapeutic strategies for inhibition of the dormant-to-proliferative metastatic switch.

Bhalla N, **Dernburg AF**. Prelude to a division. *Annual Review of Cell and Developmental Biology*, 2008 Jul 2. [Epub ahead of print] PMID: 18597662

Accurate segregation of chromosomes during meiosis requires physical links between homologs. These links are usually established through chromosome pairing, synapsis, and recombination, which occur during meiotic prophase. How chromosomes pair with their homologous partners is one of the outstanding mysteries of meiosis. Surprisingly, experimental evidence indicates that different organisms have found more than one way to accomplish this feat. Whereas some species depend on recombination machinery to achieve homologous pairing, others are able to pair and synapse their homologs in the absence of recombination. To ensure specific pairing between homologous chromosomes, both recombination-dependent and recombination-independent mechanisms must strike the proper balance between forces that promote chromosome interactions and activities that temper the promiscuity of those interactions. The initiation of synapsis is likely to be a tightly regulated step in a process that must be mechanically coupled to homolog pairing.

Downing KH, Glaeser RM. Restoration of weak phase-contrast images recorded with a high degree of defocus: The "twin image" problem associated with CTF correction. *Ultramicroscopy*, 2008 Aug; 108(8): 921-8. PMID: 18508199

Relatively large values of objective-lens defocus must normally be used to produce detectable levels of image contrast for unstained biological specimens, which are generally weak phase objects. As a result, a subsequent restoration operation must be used to correct for oscillations in the contrast transfer function (CTF) at higher resolution. Currently used methods of CTF correction assume the ideal case in which Friedel mates in the scattered wave have contributed pairs of Fourier components that overlap with one another in the image plane. This "ideal" situation may be only poorly satisfied, or not satisfied at all, as the particle size gets smaller, the defocus value gets larger, and the resolution gets higher. We have therefore investigated whether currently used methods of CTF correction are also effective in restoring the single-sideband image information that becomes displaced (delocalized) by half (or more) the diameter of a particle of finite size. Computer simulations are used to show that restoration either by "phase flipping" or by multiplying by the CTF recovers only about half of the delocalized information. The other half of the delocalized information goes into a doubly defocused "twin" image of the type produced during optical reconstruction of an in-line hologram. Restoration with a Wiener filter is effective in recovering the delocalized information only when the signal-to-noise ratio (S/N) is orders of magnitude higher than that which exists in low-dose images of biological specimens, in which case the Wiener filter approaches division by the CTF (i.e. the formal inverse). For realistic values of the S/N, however, the "twin image" problem seen with a Wiener filter is very similar to that seen when either phase flipping or multiplying by the CTF is used for restoration. The results of these simulations suggest that CTF correction is a poor alternative to using a Zernike-type phase plate when imaging biological specimens, in which case the images can be recorded in a close-to-focus condition, and delocalization of high-resolution information is thus minimized.

Taylor KA, **Glaeser RM**. Retrospective on the early development of cryoelectron microscopy of macromolecules and a prospective on opportunities for the future. *Journal of Structural biology*, 2008 Jun 19. [Epub ahead of print] PMID: 18606231

> Methods for preserving specimen hydration in protein crystals were pursued in the early 1970s as a prerequisite for protein crystallography using an electron microscope. Three laboratories approached this question from very different directions. One built a differentially pumped hydration chamber that could maintain the crystal in a liquid water environment, a second maintained hydration by rapidly freezing the protein crystal and examining it in a cold stage, and the third replaced the water of hydration by using glucose in the same way as one had previously used "negative stains". Each of these early efforts succeeded in preserving the structures of protein crystals at high resolution within the vacuum of the electron microscope, as demonstrated by electron diffraction patterns. The next breakthrough came in the early 1980s when a technique was devised to preserve noncrystalline specimens by freezing them within vitreous ice. Since then, with the development of high stability cold stages and transfer mechanisms compatible with many instrument platforms, and by using commercially provided low dose imaging techniques to avoiding radiation damage, there has been an explosion of applications. These now include single particles, helical filaments, 2-D arrays and even whole cells, where the most exciting recent applications involve cryoelectron tomography. These achievements and possibilities generate a new set of research opportunities associated with increasing the reliability and throughput with which specimens can be studied by cryoEM.

Glaeser RM. Retrospective: Radiation damage and its associated "Information Limitations". *Journal of Structural Biology*, 2008 Jun 8. [Epub ahead of print]PMID: 18588985

> The fact that radiation damage would limit the usefulness of electron microscopy with biological specimens was a concern in the earliest days of the field. Good estimates of what that limitation must be can be made by using Rose's empirical relationship between the inherent image contrast, the exposure used to record an image, and the smallest feature size that is detectable. Such estimates show that it is necessary to average many images in order to obtain statistically well-defined data at high-resolution. Structures are now routinely obtained by averaging large numbers of shot-noise limited images, and some of these extend to atomic-resolution. The signal level in current images is nevertheless far below what physics would allow it to be. A possible explanation is that beam-induced movement limits the quality of images recorded by electron microscopy. For specimens embedded in vitreous ice, beam-induced movement can even be severe enough to limit the resolution achieved during tomographic reconstruction. The fact that very high-quality images can nevertheless be obtained, although only unpredictably, suggests that it may be possible to devise new techniques of specimen preparation and/or data collection that at least partially overcome beam-induced movement. If so, the need for image averaging would be correspondingly reduced.

Sun Y, Wong N, Guan Y, Salamanca CM, Cheng JC, Lee JM, **Gray JW,** Auersperg N. The eukaryotic translation elongation factor eEF1A2 induces neoplastic properties and mediates tumorigenic effects of ZNF217 in precursor cells of human ovarian carcinomas. *International Journal of Cancer*, 2008 Oct 15;123(8):1761-9. PMID: 18661515

> Ovarian epithelial carcinomas (OECs) frequently exhibit amplifications at the 20q13 locus which is the site of several oncogenes, including the eukaryotic elongation factor EEF1A2 and the transcription factor ZNF217. We reported previously that overexpressed ZNF217 induces neoplastic characteristics in precursor cells of OEC. Unexpectedly, ZNF217, which is a transcriptional repressor, enhanced expression of eEF1A2. In our study, array comparative genomic hybridization, single nucleotide polymorphism and

Affymetrix analysis of ZNF217-overexpressing cell lines confirmed consistently increased expression of eEF1A2 but not of other oncogenes, and revealed early changes in EEF1A2 gene copy numbers and increased expression at crisis during immortalization. We defined the influence of eEF1A2 overexpression on immortalized ovarian surface epithelial cells, and investigated interrelationships between effects of ZNF217 and eEF1A2 on cellular phenotypes. Lentivirally induced eEF1A2 overexpression caused delayed crisis, apoptosis resistance and increases in serum-independence, saturation densities and anchorage independence. siRNA to eEF1A2 reversed apoptosis resistance and reduced anchorage independence in eEF1A2-overexpressing lines. Remarkably, siRNA to eEF1A2 was equally efficient in inhibiting both anchorage independence and resistance to apoptosis conferred by ZNF217 overexpression. Our data define neoplastic properties that are caused by eEF1A2 in nontumorigenic ovarian cancer precursor cells, and suggest that eEF1A2 plays a role in mediating ZNF217-induced neoplastic progression.

Chenna A, Gupta RC, Bonala RR, Johnson F, **Hang B**. Synthesis of the fully protected phosphoramidite of the benzene-DNA adduct, N2-(4-Hydroxyphenyl)-2'-deoxyguanosine and incorporation of the later into DNA oligomers. *Nucleosides Nucleotides Nucleic Acids*, 2008 Aug; 27(8):979-91. PMID: 18696366

N(2)- (4-Hydroxyphenyl)-2'-deoxyguanosine-5'-O-DMT-3'-phosphoramidite has been synthesized and used to incorporate the N(2)-(4-hydroxyphenyl)-2'-dG (N(2)-4-HOPh-dG) into DNA, using solid-state synthesis technology. The key step to obtaining the xenonucleoside is a palladium (Xantphos-chelated) catalyzed N(2)-arylation (Buchwald-Hartwig reaction) of a fully protected 2'-deoxyguanosine derivative by 4-isobutyryloxybromobenzene. The reaction proceeded in good yield and the adduct was converted to the required 5'-O-DMT-3'-O-phosphoramidite by standard methods. The latter was used to synthesize oligodeoxynucleotides in which the N(2)-4-HOPh-dG adduct was incorporated site-specifically. The oligomers were purified by reverse-phase HPLC. Enzymatic hydrolysis and HPLC analysis confirmed the presence of this adduct in the oligomers.

Chrencik JE, Brooun A, Zhang H, Mathews II, **Hura GL**, Foster SA, Perry JJ, Streiff M, Ramage P, Widmer H, Bokoch GM, **Tainer JA**, Weckbecker G, Kuhn P. Structural basis of guanine nucleotide exchange mediated by the T-cell essential Vav1. *Journal Molecular Biology*, 2008 Jul 25; 380(5):828-43. PMID: 18589439

The guanine nucleotide exchange factor (GEF) Vav1 plays an important role in T-cell activation and tumorigenesis. In the GEF superfamily, Vav1 has the ability to interact with multiple families of Rho GTPases. The structure of the Vav1 DH-PH-CRD/Rac1 complex to 2.6 A resolution reveals a unique intramolecular network of contacts between the Vav1 cysteine-rich domain (CRD) and the C-terminal helix of the Vav1 Dbl homology (DH) domain. These unique interactions stabilize the Vav1 DH domain for its intimate association with the Switch II region of Rac1 that is critical for the displacement of the guanine nucleotide. Small angle x-ray scattering (SAXS) studies support this domain arrangement for the complex in solution. Further, mutational analyses confirms that the atypical CRD is critical for maintaining both optimal guanine nucleotide exchange activity and broader specificity of Vav family GEFs. Taken together, the data outline the detailed nature of Vav1's ability to contact a range of Rho GTPases using a novel protein-protein interaction network.

Farias ST, Mungas D, Reed BR, Cahn-Weiner D, **Jagust W**, Baynes K, Decarli C. The measurement of everyday cognition (ECog): scale development and psychometric properties. *Neuropsychology*, 2008 Jul; 22(4):531-44. PMID: 18590364

This article describes the development and validation of an instrument to assess cognitively mediated functional abilities in older adults, Everyday Cognition (ECog). The ECog is an informant-rated questionnaire comprised of multiple subscales. Confirmatory factor analysis (CFA) was used to examine its

factor structure. Convergent validity was evaluated by comparing it to established measures of everyday function. External validity was evaluated by comparing ECog results across different clinical groups [cognitively normal, mild cognitive impairment (MCI), dementia]. CFA supported a seven-factor model including one global factor and six domain-specific factors (Everyday Memory, Language, Visuospatial Abilities, Planning, Organization, and Divided attention). The ECog correlated with established measures of functional status and global cognition, but only weakly with age and education. The clinical groups performed differently in each domain. In addition to the global factor, the Everyday Memory factor independently differentiated MCI from Normal, while the Everyday Language domain differentiated Dementia from MCI. Different subtypes of MCI also showed different patterns. Results suggest the ECog shows promise as a useful tool for the measurement of general and domain-specific everyday functions in the elderly.

Ju H, **Hang C**, **Andarawewa, K**, **Yaswen, P, Barcellos-Hoff, MH**, **Parvin**, **B**. Integrated profiling of cell surface protein and nuclear marker for discriminant analysis. *Biomedical Imaging: From Nano to Macro*, Jun 13, 2008, pg 1342 - 1346. ISBN: 978-1-4244-2002-5

Cell membrane proteins play an important role in tissue architecture and cell-cell communication. We hypothesize that segmentation and multivariate characterization of the distribution of cell membrane proteins, on a cell-cell basis, enable improved classification of treatment groups and identify important characteristics that can otherwise be hidden. We have developed a series of computational steps to (i) delineate cell membrane protein signals and associate them with specific nuclei, (ii) compute a coupled representation of the multiplexed DNA content with membrane proteins and other end points, (iii) evaluate computed features associated with such a multivariate representation, and (iv) discriminate between treatment groups in an optimal fashion. The novelty of our method is in the segmentation of the membrane signal and the multivariate representation of phenotypes on a cell-cell basis. To test the utility of the new method, the proposed computational steps were applied to images of cells that have been irradiated with different radiation qualities in the presence and absence of TGF?. These samples are labeled for their DNA content and E-cadherin membrane protein. We demonstrate that multivariate representation of cell-cell phenotypes improves predictive and visualization capabilities among different treatment groups, and increases quantitative sensitivity of cellular responses.

Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, **Kuo WL**, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, **Gray JW**, Bernards R, Mills GB, Hennessy BT. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Research*, 2008 Aug 1;68 (15):6084-91. PMID: 18676830

Phosphatidylinositol 3-kinase (PI3K)/AKT pathway aberrations are common in cancer. By applying mass spectroscopy-based sequencing and reverse-phase protein arrays to 547 human breast cancers and 41 cell lines, we determined the subtype specificity and signaling effects of PIK3CA, AKT, and PTEN mutations and the effects of PIK3CA mutations on responsiveness to PI3K inhibition in vitro and on outcome after adjuvant tamoxifen. PIK3CA mutations were more common in hormone receptor-positive (34.5%) and HER2-positive (22.7%) than in basal-like tumors (8.3%). AKT1 (1.4%) and PTEN (2.3%) mutations were restricted to hormone receptor-positive cancers. Unlike AKT1 mutations that were absent from cell lines, PIK3CA (39%) and PTEN (20%) mutations were more common in cell lines than tumors, suggesting a selection for these but not AKT1 mutations during adaptation to culture. PIK3CA mutations did not have a significant effect on outcome after adjuvant tamoxifen therapy in 157 hormone receptor-positive breast cancer patients. PIK3CA mutations, in comparison with PTEN loss and AKT1 mutations, were associated with significantly less and inconsistent activation of AKT and of downstream PI3K/AKT signaling in tumors and cell lines. PTEN loss and PIK3CA mutation were frequently concordant, suggesting different

contributions to pathophysiology. PTEN loss rendered cells significantly more sensitive to growth inhibition by the PI3K inhibitor LY294002 than did PIK3CA mutations. Thus, PI3K pathway aberrations likely play a distinct role in the pathogenesis of different breast cancer subtypes. The specific aberration present may have implications for the selection of PI3K-targeted therapies in hormone receptor-positive breast cancer.

Marchetti F, Cabreros D, **Wyrobek AJ**. Laboratory methods for the detection of chromosomal structural aberrations in human and mouse sperm by fluorescence in situ hybridization. *Methods in Molecular Biology*, 2008;410:241-71. PMID: 18642604

The father, like the mother, can transmit genetic defects that are detrimental for development and genetic health for his children, but the mechanisms for paternally mediated abnormal reproductive outcomes remain poorly understood. A battery of sensitive methods has been developed for detecting genetic damage associated with infertility, spontaneous abortions, as well as inherited defects in children such as aneuploidy syndromes, translocation carriers, and certain genetic diseases directly in sperm. Among these, fluorescence in situ hybridization (FISH) sperm-based assays for measuring numerical abnormalities and structural chromosomal aberrations are now available for an expanding number of species including humans, rodents, and several domesticated animals. This new generation of sperm FISH methods has identified several paternal risk factors such as age, various drugs, lifestyles, and various environmental and occupational exposures. These sperm FISH assays provide new opportunities to identify and characterize male reproductive risks associated with genetic, lifestyle, and environmental factors. This chapter outlines the laboratory methods for the detection of sperm with chromosomal structural aberrations in humans (ACM assay) and mice (CT8 assay) that have been validated for detecting environmental germ cell mutagens.

Landau SM, Lal R, **O'Neil JP**, Baker S, **Jagust WJ**. Striatal dopamine and working memory. *Cerebral Cortex*, 2008 Jun 11. [Epub ahead of print] PMID: 18550595

Recent studies have emphasized the importance of dopamine projections to the prefrontal cortex (PFC) for working memory (WM) function, although this system has rarely been studied in humans in vivo. However, dopamine and PFC activity can be directly measured with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), respectively. In this study, we examined WM capacity, dopamine, and PFC function in healthy older participants in order to test the hypothesis that there is a relationship between these 3 factors. We used the PET tracer 6-[(18)F]fluoro-L-m-tyrosine to measure dopamine synthesis capacity in the striatum (caudate, putamen), and event-related fMRI to measure brain activation during different epochs (cue, delay, probe) of a WM task. Caudate (but not putamen) dopamine correlated positively with WM capacity, whereas putamen (but not caudate) dopamine correlated positively with motor speed. In addition, delay-related fMRI activation in a left inferior prefrontal region was related to both caudate dopamine and task accuracy, suggesting that this may be a critical site for the integration of WM maintenance processes. These results provide new evidence that striatal dopaminergic function is related to PFC-dependent functions, particularly brain activation and behavioral performance during WM tasks.

Hooker JM, **O'Neil JP**, Romanini DW, **Taylor SE**, Francis MB. Genome-free viral capsids as carriers for positron emission tomography radiolabels. *Molecular Imaging and Biology*, 2008 Jul-Aug;10(4):182-91. PMID: 18437498

PURPOSE: We have developed a modular synthetic strategy to append imaging agents to a viral capsid. PROCEDURES: The hollow protein shell of bacteriophage MS2 (mtMS2) was labeled on its inside surface with [18F]fluorobenzaldehyde through a multistep bioconjugation strategy. An aldehyde functional group

was first attached to interior tyrosine residues through a diazonium coupling reaction. The aldehyde was further elaborated to an alkoxyamine functional group, which was then condensed with n.c.a. [18F]fluorobenzaldehyde. Biodistribution of the radioactive MS2 conjugates was subsequently evaluated in Sprague-Dawley rats. RESULTS: Relative to fluorobenzaldehyde, fluorine-18-labeled MS2 exhibited prolonged blood circulation time and a significantly altered excretion profile. It was also observed that additional small molecule cargo installed inside the capsids did not alter the biodistribution. CONCLUSIONS: These studies provide further insight into the pharmacokinetic behavior of nanomaterials and serve as a platform for the future development of targeted imaging and therapeutic agents based on mtMS2.

Radisky DC, **LaBarge MA**. Epithelial-mesenchymal transition and the stem cell phenotype. *Cell Stem Cell,* 2008 Jun 5; 2(6):511-2. PMID: 18522839

Epithelial-mesenchymal transition (EMT) is a developmental process in which epithelial cells acquire the motile, migratory properties of mesenchymal cells. In a recent issue of Cell, Mani et al. (2008) show that induction of EMT stimulates cultured breast cells to adopt characteristics of stem cells.

Dietrich KA, **Sindelar CV**, Brewer PD, **Downing KH**, Cremo CR, Rice SE. The kinesin-1 motor protein is regulated by a direct interaction of its head and tail. *Proceeding of the National Academy of Sciences USA,* 2008 Jul 1;105(26):8938-43. PMID: 18579780

Kinesin-1 is a molecular motor protein that transports cargo along microtubules. Inside cells, the vast majority of kinesin-1 is regulated to conserve ATP and to ensure its proper intracellular distribution and coordination with other molecular motors. Regulated kinesin-1 folds in half at a hinge in its coiled-coil stalk. Interactions between coiled-coil regions near the enzymatically active heads at the N terminus and the regulatory tails at the C terminus bring these globular elements in proximity and stabilize the folded conformation. However, it has remained a mystery how kinesin-1's microtubule-stimulated ATPase activity is regulated in this folded conformation. Here, we present evidence for a direct interaction between the kinesin-1 head and tail. We photochemically cross-linked heads and tails and produced an 8-A cryoEM reconstruction of the cross-linked head-tail complex on microtubules. These data demonstrate that a conserved essential regulatory element in the kinesin-1 tail interacts directly and specifically with the enzymatically critical Switch I region of the head. This interaction suggests a mechanism for tail-mediated regulation of the ATPase activity of kinesin-1. In our structure, the tail makes simultaneous contacts with the kinesin-1 head and the microtubule, suggesting the tail may both regulate kinesin-1 in solution and hold it in a paused state with high ADP affinity on microtubules. The interaction of the Switch I region of the kinesin-1 head with the tail is strikingly similar to the interactions of small GTPases with their regulators, indicating that other kinesin motors may share similar regulatory mechanisms.

Sudo H, Garbe J, **Stampfer MR**, Barcellos-Hoff MH, **Kronenberg A**. Karyotypic instability and centrosome aberrations in the progeny of finite life-span human mammary epithelial cells exposed to sparsely or densely ionizing radiation. *Radiation Research*, 2008 Jul;170(1):23-32. PMID: 18582160

The human breast is sensitive to radiation carcinogenesis, and genomic instability occurs early in breast cancer development. This study tests the hypothesis that ionizing radiation elicits genomic instability in finite life-span human mammary epithelial cells (HMEC) and asks whether densely ionizing radiation is a more potent inducer of instability. HMEC in a non-proliferative state were exposed to X rays or 1 GeV/nucleon iron ions followed by delayed plating. Karyotypic instability and centrosome aberrations were monitored in expanded clonal isolates. Severe karyotypic instability was common in the progeny of cells that survived X-ray or iron-ion exposure. There was a lower dose threshold for severe karyotypic instability after iron-ion exposure. More than 90% of X-irradiated colonies and >60% of iron-ion-irradiated

colonies showed supernumerary centrosomes at levels above the 95% upper confidence limit of the mean for unirradiated clones. A dose response was observed for centrosome aberrations for each radiation type. There was a statistically significant association between the incidence of karyotypic instability and supernumerary centrosomes for iron-ion-exposed colonies and a weaker association for X-irradiated colonies. Thus genomic instability occurs frequently in finite life-span HMEC exposed to sparsely or densely ionizing radiation and may contribute to radiation-induced breast cancer.

Williams PT. Independent effects of cardiorespiratory fitness, vigorous physical activity, and body mass index on clinical gallbladder disease risk. *American Journal of Gastroenterology*, 2008 Jul 10. [Epub ahead of print]PMID: 18637096

BACKGROUND AND AIMS: Incident self-reported physician-diagnosed clinical gallbladder disease was compared to BMI, body dimensions, physical activity (km/day run) and cardiorespiratory fitness (10 km race speed, meters per second [m/s]) in 29,110 male and 11,953 female runners. METHODS: Physician-diagnosed gallbladder disease was reported by 166 men (0.57%) and 112 women (0.94%) during (mean +/- SD) 7.74 +/- 1.84 and 7.42 +/- 2.10 years of follow-up, respectively. RESULTS: There was a progressive increase in age-adjusted risk with increasing BMI that accelerated sharply above 27.5 kg/m(2). Even among ostensibly healthy-weight women, the age-adjusted risk was significantly greater above 22.5 kg/m(2) vis-à-vis the leanest women (P= 0.04). Age-adjusted risk declined with increasing fitness in both sexes. Compared to the least fit men and women, men who ran faster than 4.75 m/s had 83% lower risk (75% lower when adjusted for km/day and BMI) and women who ran faster than 4 m/s had 93% lower risk (85% lower adjusted for km/day and BMI). The fittest men (>/=4.75 m/s) were at significantly less risk than men who ran <3.25 m/s (P < 0.003) and between 3.25 and 3.75 m/s (P= 0.03), and the fittest women (>/=4 m/s) were at significantly less risk than those who ran <2.8 m/s (P < 0.0001), between 2.8 and 3.2 (P= 0.0004), 3.2 and 3.6 (P= 0.002), and 3.6 and 4.0 m/s (P= 0.005). Adjustment for BMI accounted for more of the risk reduction associated with fitness in women than men. The risk for clinical gallbladder disease was also significantly related to usual running distance (men: P= 0.01; females: P= 0.008), which was attributable to the leanness of the longer-distance runners. CONCLUSION: Clinical gallbladder disease risk was (a) concordantly related to BMI, (b) inversely related to usual running distance, and (c) inversely related to cardiorespiratory fitness independent of physical activity levels.